2007 (2008). The Journal of Arachnology 35:487–492

JUMPING SPIDERS ASSOCIATE FOOD WITH COLOR CUES IN A T-MAZE

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ABSTRACT. Salticid spiders are a tractable group for studies of learning. We presented *Phidippus princeps* Peckham & Peckham 1883 with the challenging task of associating prey with color cues in a T-maze. Experimental spiders were given the opportunity to learn that a cricket was hidden behind a block of a particular color. To eliminate the use of other cues, we randomly assigned both block position within the maze, and maze location within the room. For control spiders, no cues predicted the location of prey. We gave spiders two blocks of trials. Each block consisted of four training trials followed by a probe trial in which no prey was present. Trials lasted an hour, and spiders were given one trial per day. Not all spiders were successful in finding the prey during training trials. In the first probe trial, there was no evidence of learning: there was no effect of treatment, the number of successful training trials, or their interaction on which block the spiders chose first. In the second probe trial, there was a significant interaction between treatment and number of successful training trials: experimental-group spiders with a greater number of successful training trials were more likely to choose the correct block in the probe trial. This study demonstrates that *P. princeps* can learn the location of prey by color cues alone, a challenging task, and adds to the growing literature on learning in spiders.

Keywords: Learning, experience, *Phidippus*, Salticidae, vision

In spite of their small brain size, many spider species are capable of modifying their behavior with experience. Experience influences behavior in many aspects of spiders' lives, including mate choice (Hebets 2003), foraging (Jackson & Wilcox 1993; Sebrier & Krafft 1993; Edwards & Jackson 1994; Punzo 2002a, b; Punzo & Ludwig 2002; Nakata et al. 2003), antipredator behavior (Punzo 1997), locomotory behavior (Punzo & Alvarez 2002) and intraspecific conflict (Whitehouse 1997; Dodson & Schwaab 2001; Hoefler 2002).

Jumping spiders (Araneae, Salticidae) are an exceptionally good model system for studying learning. They are renowned for the visual acuity of their anterior median eyes (Land & Nilsson 2002), so visual stimuli are likely to be salient and easily sensed. Visual stimuli are also relatively easy to standardize compared to other sensory modalities such as odor, so this system is particularly tractable experimentally.

Jumping spiders have been shown to be able to learn in several different contexts. Most species are generalist predators, so it would be beneficial to have the ability to learn to avoid dangerous or distasteful prey, or to select beneficial prey. Even when prey toxins are not fatal, predators that feed repeatedly on them may sicken and grow more slowly (Paradise & Stamp 1991; Strohmeyer et al. 1998; Toft 1999). Salticid species do indeed learn during foraging. For example, with experience, *Phidippus regius* Koch 1846 improve in their ability to capture palatable prey and decline in their tendency to attack ants. Naive *P. princeps* Peckham & Peckham 1883 readily attack milkweed bugs, but learn to avoid them after repeated exposures (Skow & Jakob 2006).

Learning also has clear fitness consequences during navigation. *Phidippus* spp., like many other jumping spiders, construct a silken nest for protection during the night, in inclement weather, and during egg guarding (Jackson 1979). Spiders often forage away from their nests during the day, but return to them at dusk. *Phidippus clarus* Keyserling 1885 apparently attends to cues near its nests in order to locate it again: spiders that had nests on wooden dowels were more likely to approach novel dowels of the same color than were either spiders with no experience with dowels, or with experience only with dowels of a different color (Hoefler & Jakob 2006).

We presented *Phidippus princeps* with the task of moving through a simple maze in order to find prey associated with a cue of a particular color. Only cue color and not its location predicted the location of prey. This task was potentially challenging: in nature, rewards may be associated with reliable cues (e.g., nest sites associated with particular structural characteristics, or prey associated with flowers or dung pats), but the cues are likely to be stable in location. However, Hoefler & Jakob's (2006) experiment, described above, demonstrated that *P. clarus* can recognize nest sites based solely on color cues: spiders had equivalent levels of response to familiar beacons (both color and location cues present) and novel beacons of the same color (color cues present but location cues absent). We were interested in whether the spiders could learn a similar task in the context of foraging.

A second feature of the current experiment is that we gave spiders only a single training trial per day, so they were required to remember the association for a long period. Again, this makes this a challenging task, but perhaps this time delay between training trials is similar to what foraging spiders encounter in nature. Given that *P. princeps* live in heterogeneous habitats with patchily-distributed prey and vegetation types, spiders may not encounter a particular pairing of prey with a particular environmental feature very frequently.

METHODS

Spider collection and maintenance.—We captured *P. princeps* spiders by sweep netting fields with a mixture of grasses and wildflowers in Amherst, MA, in the fall of 2003. Voucher specimens have been placed in the entomology collection at the University of Massachusetts Amherst. We kept spiders individually in ventilated plastic cages, either 23 × 31 × 10 cm high or 13.5 × 19 × 9.5 cm high, and provided ad libitum water and 4–6 crickets weekly. Each cage contained a painted green stick and leafy plastic vines to encourage normal spider behavior (Carducci & Jakob 2000). The daily light cycle was 14L:10D.

Testing.—The apparatus and procedure closely followed Popson (1999). Adult females were tested during winter 2003–04. We constructed T-mazes of 6 mm thick Plexiglas. The entry arm of the maze was 30 cm long, and the top of the T measured 40 cm from tip to tip. All arms were 10 cm wide × 10 cm high. We covered the outside walls with white contact paper to reduce visual cues from the room. A thin film of petroleum jelly lining the bottom of the inner walls discouraged spiders from climbing.

Cues were wooden cubes 3.2 cm on a side, painted either red or blue (Aleene's Premium Coat Acrylic, True Red: reflectance peak at 700 nm; Deep Blue: reflectance peak at 450 nm). These colors were chosen because spiders could distinguish similar colors in a previous experiment (Popson 1999). Colors were not matched for saturation or brightness. We coated the blocks in petroleum jelly to discourage spiders from climbing on them. We placed one block at each end of the top of the T, 5.5 cm from the end wall. The reward was a live cricket secured with nontoxic glue to a small piece of index card. In preliminary tests, spiders readily fed on crickets prepared in this way. Spiders could see the cricket only by walking behind the block.

We randomly assigned 55 spiders to either a control or experimental group. In the control group's training trials, prey were placed randomly with respect to both side of the maze (left or right) and color of block (red or blue). In the experimental group, prey were placed randomly with respect to side, but were always behind the same color block. The rewarded color was assigned randomly for each individual. For both groups, location of the maze was assigned randomly for each trial, so that room cues (such as direction of the light source or appearance of the ceiling) did not indicate prey location. Thus, for the experimental group, only block color predicted prey location, and for the control group, no cues predicted prey location.

We placed mazes on top of a layer of sand inside a 2-m^2 arena. The sand reduced vibrations that may have disturbed the spiders. Seven mazes were run simultaneously. We released spiders into the mazes via a 20 ml, 2-cm diameter open-topped syringe, covered with opaque tape. We placed a spider into a syringe, blocked the top with a cotton ball wrapped in a tissue, and inserted the syringe into a hole drilled through the wall in the center of the bottom of the T. Between trials, syringes and mazes were washed with soapy water, sprayed with alcohol and wiped dry to disrupt any chemical cues left by previous spiders. Fresh cotton plugs were used for each trial.

Spiders were allowed to acclimate in the syringe for 5–8 min before the start of the trial. We then started the video camera mounted above the mazes, moved to the first maze, removed the cotton ball, and slowly pushed the syringe plunger flush with the inner wall of the maze. We moved swiftly from maze to maze so that all trials began within 2 min, and then left the room so that spiders were not disturbed. Trials were terminated after an hour, and spiders were removed from their mazes in the same order in which they were put in. Review of videotapes revealed that no spider finished feeding on a prey prior to the end of the hour.

We tested spiders once per day for 10 consecutive days between 10:00 and 15:00 h. Days 1–4 and 6–9 were training trials, and prey were present in the maze. Days 5 and 10 were probe trials with the same procedure but with no prey in order to eliminate the effect of odor or sound cues from the prey on the spiders' choices.

Not all spiders captured prey during training trials, so not all spiders had equal opportunity to learn the task. We scored a training trial as successful if the spider fed on the prey. For experimental spiders, we scored a probe trial as correct if the spider walked behind the rewarded block first. For control spiders, at the start of the experiment we randomly assigned a block color for each spider. We scored a probe trial as correct if the spider walked behind the randomly assigned block. We chose this method instead of assigning a particular color to be correct in case spiders were more likely to favor a particular color; however, the analyses generated indistinguishable results.

Analysis.—We used logistic regression, with the choice of block in the test trial as the dependent variable. We tested three independent variables: treatment group, the number of successful training trials (a continuous variable), and their interaction. If experience influences choice in the probe trial, spiders in the experimental group, where color predicted prey location, should improve with higher numbers of successful training trials. In control spiders, there should be no relationship between the number of successful trials and choice in the probe trial. Thus, we expected a significant interaction term if learning took place.

RESULTS

There was no effect of treatment, training, or their interaction on the outcome of the probe trial on Day 5 (Table 1). Thus, we have no evidence that spiders learned the association in the first four training trials.

Approximately equal numbers of spiders in the control and experimental groups chose the correct block in Trial 10 (control: 10 of 22; experimental: 13 of 23). However, there was a significant interaction between training success and group: the number of successful training trials (out of a total of eight training trials) increased the probability of experimental spiders finding the prey in the test trial, but not that of control spiders (Fig. 1; Table 1). Another way to examine this question is to compare the number of successful training trials for spiders that made correct vs. wrong choices. Experimental spiders that made the correct choice in probe Trial 10 had significantly more successful training trials than spiders that made the wrong choice (unpaired t-test; $t = 2.339$; $P < 0.03$; mean ± SE, correct: 5.5 ± 0.49, incorrect: 3.7 ± 0.63). However, there was no difference for control spiders ($t = -1.291$; $P = 0.19$, correct: 3.0 ± 0.63; incorrect: 4.3 ± 0.71).

DISCUSSION

Phidippus princeps jumping spiders were significantly more likely to look behind a block that visually predicted the presence of prey when they had an adequate number of successful training trials to gain this experience. Spiders showed no evidence of learning after four training trials, and even after eight training trials a substantial number of spiders made the wrong choice in the probe trial.

Table 1.——Success of spiders in probe trials on days 5 and 10. For probe trials on Day 5, there was a maximum of four successful training trials. For probe trials on Day 10, there was a maximum of eight successful training trials.

	df	Likelihood Ratio χ^2	*P*
Probe trial on Day 5			
Group (control vs. experimental)	1	0.620	0.43
Number of successful training trials	1	2.515	0.11
Group × number of successful training trials	1	0.205	0.65
Probe trial on Day 10			
Group (control vs. experimental)	1	0.261	0.61
Number of successful training trials	1	0.680	0.41
Group× number of successful training trials	1	6.661	0.01

Jumping spiders have UV, green, and possibly blue and even red-sensitive cells in the retina of their anterior median eyes (reviewed in Lim & Li 2006). Thus, it is possible that our spiders used hue to distinguish between the red and blue cues that we presented. However, because we did not control for brightness or saturation of our color cues, we cannot be certain that spiders relied solely on hue.

The learning task was particularly difficult for a number of reasons. First, because of the length of time required for each trial, we ran multiple trials simultaneously. In order to avoid disturbing spiders, we left all spiders in their mazes for one hour rather than removing them after they made their initial choices. This meant that a spider could make an incorrect choice, and indeed spend much of its time on the unrewarded side of the maze, but then make the correct choice and capture the prey. Thus, the strength of the relationship between the stimulus and the reward was low. In contrast, in a number of other studies, spiders were given more extensive experience with the task to be learned (e.g., Punzo 2002a; Punzo & Preshkar 2002; Hoefler & Jakob 2006).

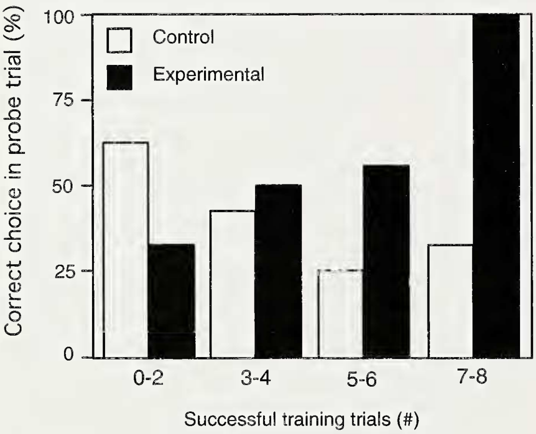

Figure 1.—The—percentage of *Phidippus princeps* that chose the correct block in the second probe trial after two blocks of four training trials. Not all spiders successfully found and attacked the prey on all training trials. For the experimental group, where prey were always hidden behind the same color block, increased success in training trials led to better performance at the probe trial. There was no relationship for control spiders, for which no cues consistently predicted the location of prey.

This learning task was also difficult because the time between the final training trial and the probe trial was quite long (24 h). In most controlled studies of associative learning in spiders, only much shorter retention periods have been examined. For example, Skow & Jakob (2006) trained spiders to avoid aversive prey, and then tested them 50 min after their final training trial. In another experiment, Skow (2007) gave spiders a series of electric shocks paired with a set of visual cues. Five min after the completion of the training session, spiders were given a choice between shock-associated cues and another set, and significantly more often chose the new set. Nakamura & Yamashita (2000) trained jumping spiders (*Hasarius adansoni* Audouin 1826) to avoid the heated side of a small dish over a three-min session, and tested them immediately after training. Rodríguez & Gamboa (2000) found that three web-building species form memories of captured prey, and return to search for stolen prey soon after it was removed from the web. Tarantulas learned to avoid shock by raising their legs, and retained this memory for an hour (Punzo 1988). There are, however, some studies that examined longer retention periods. For example, Hebets (2003) demonstrated that female wolf spiders (*Schizocosa uetzi* Stratton 1997) exposed to courting males as subadults prefer males of the same phenotype when tested 11 days later or more after they had molted to maturity. Punzo (1997 and pers. comm.) found that wolf spiders (*Schizocosa avida* Walckenaer 1837) avoided scorpion cues 48 h after a negative encounter with a scorpion. Research that methodically examines the rate of acquisition of learned associations and the rate of decay of these memories would be especially valuable in understanding the extent to which spiders rely on learning in their daily lives.

ACKNOWLEDGMENTS

We are grateful for the assistance of Angela Nichols. AP and she were supported by the UMass Commonwealth College with Sophomore Year Fellowships. Arnold Well of the UMass Psychology Department and Michael Sutherland of the UMass Statistical Consulting Center provided statistical expertise. Karen Hollis kindly provided advice on experimental design. We thank Dan Papaj for running the spectral analyses of the cubes. Amelia Sabadini, Julie Bednarski, Ted Stankowich, two anonymous reviews, and Gail Stratton provided helpful comments on the manuscript. This project was partially supported by a NSF Doctoral Dissertation Improvement Grant to CDS, an NSF SGER grant to EMJ, and a UMass Faculty Research Grant to EMJ.

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Manuscript received 26 November 2006, revised 7 June 2007.